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Mutation Analysis of the HFE Gene Associated With Hereditary Hemochromatosis in African Americans

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Homozygosity for the mutation Cys282Tyr in the HFE gene has recently been identified as a cause of hereditary hemochromatosis, a disorder resulting in the inappropriate absorption of iron. Approximately 10% of Caucasians are heterozygous for this mutation; however, the gene frequency in African Americans is unknown. A study of a control population of African Americans was performed to determine the frequency of the Cys282Tyr and His63Asp alleles in this ethnic group. The carrier frequency for each mutant allele in our African American population was 3.0%. DNA studies of four African-American hemochromatosis patients did not identify any individuals with the Cys282Tyr allele. These findings suggest that if the Cys282Tyr mutation confers susceptibility to hemochromatosis in Caucasians (as suggested by recent studies) there is an alternative mechanism for hemochromatosis in the American black population. Am. J. Hematol. 58:213–217, 1998. © 1998 Wiley-Liss, Inc.

Key words: hemochromatosis; HFE; African Americans; mutation; carrier frequency

INTRODUCTION

Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism resulting in the accumulation of excess iron in a variety of organs. It is one of the most common inherited diseases among individuals of northern European descent, affecting between 1 in 200 [1,2] and 1 in 400 individuals [3]. Recently, two point mutations of HFE, an HLA-linked gene on the short arm of chromosome 6, were identified in patients with HH [3]. The G to A transition mutation, which occurs at nucleotide 845 of the open reading frame of HFE, results in a cysteine to tyrosine substitution at amino acid 282. Early studies identified this mutation, designated Cys282Tyr (845G → A; 845A; C282Y), on 85% of hemochromatosis chromosomes [3–7]. These studies were performed on hemochromatosis patients without reference to family history. A recent study of patients with a positive family history of hemochromatosis identified the Cys282Tyr mutation in homozygous form in all patients [5]. This study suggested that hereditary hemochromatosis results from the Cys282Tyr mutation and that the variation in the mutation frequency in previous studies was due to the heterogeneity of iron overloading disorders and/or the misclassification of hemochromatosis heterozygotes as homozygotes.

A second mutation in the HFE gene changes a histidine at position 63 to an aspartic acid, although its role in HH is controversial [3–6,8]. This mutation, designated His63Asp (187C → G; 187G; H63D) is in complete linkage disequilibrium with the Cys282Tyr mutation [4], meaning that no chromosome carries both mutations. The raw population frequency of His63Asp is similar among normal and affected chromosomes [3,6,8]. The penetrance of the compound heterozygous state is approximately 1% [3,4], suggesting that this mutation may cause disease in a patient with the Cys282Tyr mutation on the other chromosome no. 6, although at a reduced penetrance.

Previous studies of the incidence of clinical HH and the HFE mutations have focused exclusively on Caucasian populations, where the carrier frequency for the

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TABLE I. Summary of Five African American Hemochromatosis Patients Compared With Diagnostic Criteria [14]*

		Patients				
	Diagnostic criteria	1	2	3	4	5
Sex		M	M	M	F	M
Age at diagnosis		69	67	42	67	57
Transferrin saturation	>62%	85%	97%	97%	81%	85%
Serum ferritin	>1,000 μg/L	1,502	>2,500	1,743	922	4,639
Serum iron	>200 μg/dL	294	212	321	221	175
Total iron binding capacity	<200 μg/dL	347	219	332	272	206
[Iron] in liver biopsy	>10,000 μg/g dry weight	38,186	NR	NR	6,152	18,674
Cys282Tyr	+/+	−/−	−/−	−/−	−/−	ND
His63Asp	−/−	+/−	−/−	−/−	−/−	ND

*NR, not reported; ND, not determined.

Cys282Tyr mutation has been reported for persons of mixed European descent as 6.4% [3] and 15% [4] and the frequency for the His63Asp mutation has been reported as 17% for both the control and hemochromatosis patient population [3]. There is no reported information on the prevalence of these mutations within the American black population. Our analysis of a control population and African American patients with hemochromatosis revealed a lower frequency of these mutations than reported in Caucasians.

METHODS

Human Subjects and Diagnosis

Control allele frequencies were studied from archived genomic DNA samples from 172 normal, healthy unrelated African Americans. Hospital records were used to verify the ethnic background of each individual. The samples were then numbered consecutively and stored anonymously. Five African Americans were previously diagnosed with hemochromatosis at our institution (Table I). Clinical diagnosis was established by the absence of a known cause of secondary iron overload and the presence of at least two of the following criteria: (1) transferrin saturation >60%, (2) serum ferritin >1,000 μg/L; (3) serum iron >200 μg/dL; (4) total iron binding capacity <200 μg/dL (Table I). All five patients had a liver biopsy, and the liver iron concentration was reported in three patients (Table I). Three patients had cirrhosis; one of these also had skin pigmentation changes typical of this disorder. All five patients were treated with phlebotomies until either serum ferritin was at the lower end of normal or slightly below or until the patient developed mild iron-deficiency anemia. None of the patients have a family history of hemochromatosis. Four of the five African American patients were available for genotyping. HLA typing was not performed. Specimens analyzed in the current study were obtained in accordance with the guidelines approved by the Institutional Review Board at Henry Ford Hospital.

Genotyping

The primers for polymerase chain reaction (PCR) amplification of the Cys282Tyr mutation were C282Y-forward (5′ TGG CAA GGG TAA ACA GAT CC 3′) and C282Y-reverse (5′ CTC AGG CAC TCC TCT CAA CC 3′) [3]. Primers for the His63Asp mutation were H63D-forward (5′ACA TGG TTA AGG CCT GTT GC 3′) and H63D-reverse (5′ GCC ACA TCT GGC TTG AAA TT 3′) [3]. The reaction conditions for both mutations were 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM of each of dNTPs, 0.6 μM each primer, 2.5 U Taq polymerase (Promega, Madison, WI), and 200 ng genomic DNA. Cycling conditions for PCR were an initial 5 min at 95°C, followed by 38 cycles at 96°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min with a final extension time of 7 min at 72°C. The PCR product (26 μl) was digested with either 10U of *Rsa* I (Promega) (Cys282Tyr) or 10U *Mbo* I (Promega) (His63Asp) in 1 × buffer for 3–24 h. Electrophoretic separation was done using a 10% polyacrylamide gel in 1 × TBE buffer (85 mM TRIS, 90 mM boric acid, 2 mM EDTA) at 230 V for 2 h. Following staining in ethidium bromide (0.05 mg/100 ml), the separation was directly visualized by UV fluorescence.

RESULTS

The 388 bp PCR product containing the Cys282Tyr mutation of the HFE gene was amplified for each DNA specimen. Restriction digestion with *Rsa* I results in 2 fragments (248 and 140 bp) for the wild-type genotype and 3 fragments (248 bp, 111 bp [and 29 bp not visible in Fig. 1]) for the homozygous mutant (Fig. 1a) because the G → A mutation creates a new *Rsa* I recognition site. The heterozygote genotype is characterized by the presence of the 248, 140, and 111 bp fragments [and 29 bp fragment not visible in Fig. 1] (Fig. 1a). The completeness of digestion is controlled with the invariant 248 bp fragment observed in all genotypes. In a sample of 172 unrelated individuals of African American descent, we

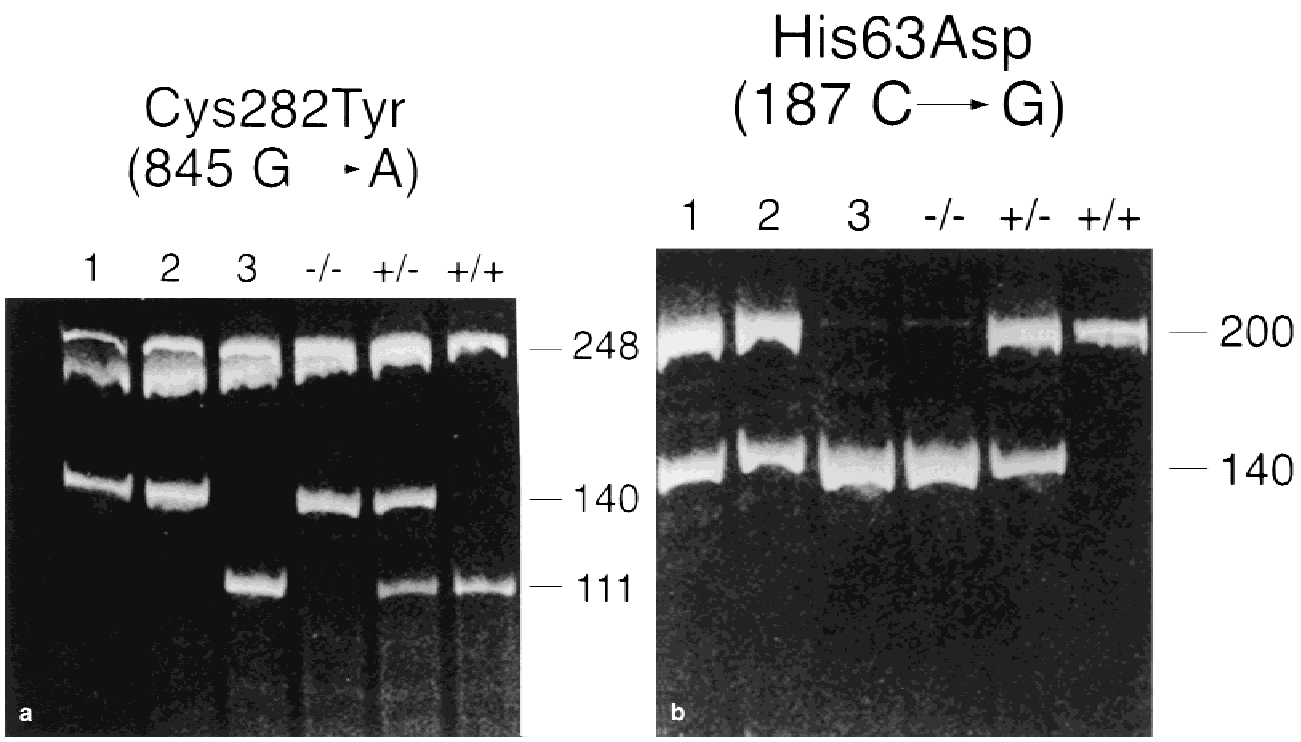

Fig. 1. a: Ethidium bromide stained polyacrylamide gel of the PCR product amplified with the primers Cys282Tyr-forward and Cys282Tyr-reverse and digested with *Rsa* I. Lanes 1 and 2: *Rsa* I digest of the homozygous normal genotype; lane 3: *Rsa* I digest of a homozygous mutant (Caucasian HH patient); lane −/−: negative control; lane +/−: heterozygous control; lane +/+: homozygous mutant control. b: Ethidium bromide stained polyacrylamide gel of the PCR product amplified with the primers His63Asp-forward and His63Asp-reverse and digested with *Mbo* I. Lanes 1 and 2: *Mbo* I digest of the heterozygous genotype; lane 3: *Rsa* I digest of a homozygous normal; lane −/−: negative control; lane +/−: heterozygous control; lane +/+: homozygous mutant control.

identified 167 individuals homozygous for the wild-type allele, 5 heterozygous for the mutant allele, and none homozygous for the mutant allele (Table II). The carrier frequency of the Cys282Tyr mutant allele in our African American population was 3.0% (therefore the mutant allele frequency was 1.5%) with a 95% confidence interval between 0.2 and 2.7%. Applying the χ^2 test using an expected carrier frequency of 6.4% [3] gave a value of 3.43 ($P = 0.064$). Applying the χ^2 test using an expected carrier frequency of 15% [4] gave a value of 18.6 ($P = 0.00002$). These data provide evidence for a difference between the incidence of the Cys282Tyr mutation in the African American and Caucasian populations, suggesting that the prevalence of the common mutation causing HH in whites is much less frequent among individuals of African descent.

The 200 bp PCR product containing the His63Asp mutation of the HFE gene was amplified for each DNA specimen. Restriction digestion with *Mbo* I results in 2 fragments (140 bp [and 60 bp not visible in Fig. 1]) for the wild-type genotype and 1 fragment (200 bp) for the homozygous mutant (Fig. 1b) because the C → G mutation destroys an *Mbo* I recognition site. The heterozygote genotype is characterized by the presence of the 200 and 140 bp fragments [and 60 bp fragment not visible in Fig. 1] (Fig. 1b). In a sample of 99 unrelated African-American individuals, we identified 96 individuals who were homozygous for the wild-type allele, 3 heterozygous for the mutant allele, and none homozygous for the mutant allele (Table III). The carrier frequency of the His63Asp mutant allele in our African-American population was 3.0% (therefore the mutant allele frequency was 1.5%) with a 95% confidence interval between 0.0 and 3.2%. Applying the χ^2 test using an expected carrier frequency of 17% [3] gave a value of 36.5 ($P < 0.0001$). Therefore, there is also a significant difference between the frequency of the mutant His63Asp allele between Caucasians and African Americans.

At our institution, a total of 61 patients have been diagnosed with and treated for primary hemochromatosis within the last 2 years. Of these patients 53 (86.9%) are Caucasian, five (8.2%) are African American, and three (4.9%) are Asian. Of the African American patients, four were available for genotyping studies. The Asian patients were not analyzed. The Cys282Tyr mutation was not identified on any of the patient chromosomes and the His63Asp mutation was identified in the heterozygous state in a single patient (Table I). Assuming no difference

TABLE II. African American Population Study of the Cys282Tyr Mutation of the HFE Gene*

Genotypes	Homozygous normal	Heterozygote	Homozygous mutant
Frequency of mutant Cys282Tyr allele		.015	
Frequency of normal allele		.985	
Observed genotype (n = 172)	167.0	5.0	0
Expected (carrier frequency of 6.4%) ($P = 0.064$)[a]	161.1	10.7	0.18
Expected (carrier frequency of 15.0%) ($P = 0.00002$)[b]	147.1	23.9	0.97

*Total numbers of genotypes and allelic frequencies.
[a]Based on the carrier frequency in the Caucasian population [3].
[b]Based on the carrier frequency in the Caucasian population [4].

TABLE III. African American Population Study of the His63Asp Mutation of the HFE Gene*

Genotypes	Homozygous normal	Heterozygote	Homozygous mutant
Frequency of mutant His63Asp allele		.015	
Frequency of normal allele		.985	
Observed genotype (n = 99)	96.0	3.0	0
Expected (carrier frequency of 34%) ($P < 0.0001$)[a]	68.2	27.9	2.9

*Total numbers of genotypes and allelic frequencies.
[a]Based on the carrier frequency in the Caucasian population [3].

in the frequency of the Cys282Tyr mutation between Caucasian and African American hemochromatosis patients, the probability that the four black patients would not have this mutant allele is $P = 6.89 \times 10^{-8}$. These results confirm that the prevalence of the Cys282Tyr mutation is different in African American hemochromatosis patients compared with Caucasian hemochromatosis patients.

DISCUSSION

The frequency of the Cys282Tyr and His63Asp mutations in the control African American population is considerably lower than the reported frequencies in the white population. Presumably the mutant alleles that are found represent admixture with the Caucasian population and are consistent with data from other genetic studies that show that the proportion of Caucasian genes in the African American population is approximately 25% [9]. These mutations may have occurred only recently in the northern European founding population. It would be interesting to estimate the admixture with the use of polymorphic markers that have been previously typed in ethnically defined populations and that have been reported to have large allele-frequency differentials [10].

By screening for HFE mutations in individuals of African American descent with primary hemochromatosis, we hoped to provide additional evidence regarding the prevalence of HH in this population. None of the African American patients studied carried the Cys282Tyr mutation and one was heterozygous for the His63Asp allele. These results suggest that African American hemochromatosis patients have a significantly lower frequency of the HFE mutations compared with Caucasian patients. It is interesting that a recent analysis of the HFE gene in Italian hemochromatosis patients identified the Cys282Tyr mutation in 69% of Italian HH chromosomes and 1% of normal chromosomes, suggesting additional genetic mechanisms for HH patients of Italian vs. northern Europe descent [8].

Recently, a largely uncharacterized syndrome called African iron overload (AIO) was recognized as clinically distinct from Caucasian hemochromatosis [11]. Differences between AIO and HH include different patterns of iron deposition in the liver and other organs. Also, transferrin saturation in HH patients becomes elevated at an early age, while transferrin saturation in patients with AIO is normal until after the development of cirrhosis [11]. AIO occurs in approximately 10% of men from sub-Saharan Africa. This disorder is associated with hepatomegaly or cirrhosis, ascorbic acid deficiency, and osteoporosis, while less common complications are heart failure and diabetes [11]. Studies have suggested that AIO is due to the interaction of an inherited factor distinct from an HLA-linked locus and excess dietary iron [12,13]. Because this syndrome is relatively newly described, it is difficult to distinguish AIO from HH in the hemochromatosis patient of African American descent. It would be important to screen patients previously described as having AIO for the hemochromatosis mutant alleles.

In conclusion, our results demonstrate that the mutations causing hemochromatosis in Caucasians are significantly less prevalent in both the African American con-

trol and hemochromatosis patient population, suggesting that hemochromatosis in African Americans may be due to either a mutation in a gene other than HFE or to an as yet unidentified mutation in the HFE gene unique to this population.

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